Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biological Control 27 (2003) 349–356
Biological Control
www.elsevier.com/locate/ybcon

Stage-specific mortality of *Rhagoletis indifferens* (Diptera: Tephritidae) exposed to three species of *Steinernema* nematodes

Wee L. Yee* and Lawrence A. Lacey

USDA-ARS, Yakima Agricultural Research Laboratory, 5230 Konnowac Pass Road, Wapato, WA 98951, USA

Received 12 November 2002; accepted 23 January 2003

Abstract

Mortality of larval, pupal, and adult western cherry fruit fly, *Rhagoletis indifferens* (Tephritidae) exposed to the steinernematid nematodes *Steinernema carpocapsae*, *Steinernema feltiae*, and *Steinernema intermedium*, was determined in the laboratory and field. Larvae were the most susceptible stage, with mortality in the three nematode treatments ranging from 62 to 100%. *S. carpocapsae* and *S. feltiae* were equally effective against larvae at both 50 and 100 infective juveniles (IJs)/cm^2. *S. intermedium* was slightly less effective against larvae than the other two species. Mortalities of *R. indifferens* larvae at 0, 2, 4, and 6 days following their introduction into soil previously treated with *S. carpocapsae* and *S. feltiae* at 50 IJs/cm^2 were 78.6, 92.5, 95.0, and 77.5% and 87.5, 52.5, 92.5, and 70.0%, respectively, and at 100 IJs/cm^2 were 90.0, 92.0, 100.0, and 84.0% and 90.0, 50.0, 42.0, and 40.0%, respectively. There was no decline in mortality caused by *S. carpocapsae* as time progressed, whereas there was in one test with *S. feltiae*. Larval mortalities caused by the two species were the same in a 1:1:1 vermiculite:peat moss:sand soil mix and a more compact silt loam soil. In the field, *S. carpocapsae* and *S. feltiae* were equally effective against larvae. Pupae were not infected, but adult flies were infected by all three nematode species in the laboratory. *S. carpocapsae* was the most effective species at a concentration of 100 IJs/cm^2 and infected 11–53% of adults that emerged. The high pathogenicity of *S. carpocapsae* and *S. feltiae* against *R. indifferens* larvae and their persistence in soil as well as efficacy in different soil types indicate both nematodes hold promise as effective biological control agents of flies in isolated and abandoned lots or in yards of homeowners.
Published by Elsevier Science (USA).

Keywords: *Rhagoletis indifferens*; *Steinernema carpocapsae*; *Steinernema feltiae*; *Steinernema intermedium*; Entomopathogenic nematode; Stage-specific mortality

1. Introduction

The western cherry fruit fly, *Rhagoletis indifferens* Curran (Tephritidae), is the major pest of sweet cherries, *Prunus avium* (L.), in the Pacific Northwest of the United States (Frick et al., 1954). Insecticides are highly effective against the fly (Zwick et al., 1970, 1975), but because of their potential loss due to implementation of the Food Quality Protection Act of 1996 and the hazards associated with their use near homes, the development of alternative, more environmentally safe control measures is needed. One such measure is the use of steinernematid nematodes against fly stages in the soil (Patterson Stark and Lacey, 1999). At present, most *R. indifferens* problems in Washington State originate from abandoned lots or unmanaged yards of homeowners. In such situations, the use of steinernematid nematodes is a feasible control alternative to insecticide use because of the small areas that need to be treated. Steinernematid nematodes are currently being used in the field against a wide variety of fruit, vegetable, turfgrass, and ornamental insect pests (Shapiro-Ilan et al., 2002) and are highly effective after they penetrate insects, releasing bacterial symbionts that cause death within 3 days (Poinar, 1990).

Rhagoletis indifferens is a univoltine insect that occurs as a larva inside cherry fruit from June to July. After

* Corresponding author. Fax: 1-509-454-5646.
E-mail address: wlyee@yarl.ars.usda.gov (W.L. Yee).

1049-9644/03/$ - see front matter. Published by Elsevier Science (USA).
doi:10.1016/S1049-9644(03)00029-X

leaving the fruit, larvae drop onto and enter the soil where they pupate (Frick et al., 1954). The larvae are probably the most vulnerable stage to nematode infection. Several nematode species were effective in killing *R. indifferens* larvae in petri plate-filter paper bioassays. *S. carpocapsae* (Weiser) and *S. feltiae* (Filipjev), which caused 70–83% mortality, were slightly more effective than two *Heterorhabditis* spp., which caused 40–62% mortality (Patterson Stark and Lacey, 1999). These promising results indicated that further evaluations of these *Steinernema* species against *R. indifferens* larvae were warranted.

The majority of the 1-year life cycle (about 10 months, beginning in mid-June) of *R. indifferens* is spent as a pupa 0–10 cm below the soil surface (AliNiazee, 1974; Frick et al., 1954). Nematodes have never been tested against *R. indifferens* pupae, but pupae of the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Tephritidae), were infected by *S. carpocapsae*,[1] although at a much lower rate than other fly stages (Beavers and Calkins, 1984). Adult *R. indifferens* are also found in the soil (presumably for <1 day) after they emerge from puparia during June, a time when they may be vulnerable to nematode infection. However, no study has tested nematodes against adult *R. indifferens*. In laboratory tests, *S. carpocapsae*[1] caused high mortality of adult *A. suspensa* (Tephritidae) (Beavers and Calkins, 1984), and it also killed adult Mediterranean fruit fly, *Ceratitis capitata* (Tephritidae) (Wiedemann) (Lindegren and Vail, 1986).

In this study, the main objective was to determine and compare the stage-specific mortality of *R. indifferens* exposed to *S. carpocapsae* and *S. feltiae* under conditions mimicking those in the field. A third species, *S. intermedium* (Poinar), was also tested against larvae and adults.

2. Materials and methods

2.1. Source of flies and general laboratory experiments

For all laboratory experiments, *R. indifferens* larvae were obtained from infested sweet and sour cherries, *P. avium L.* and *Prunus cerasus* L., respectively. Fruits were collected in June and July 2000, 2001, and 2002 from infested trees in central Washington State and placed on wire screens over tubs to collect larvae. For larval experiments, larvae dropped into water, where they remained at 15–25 °C for 0–18 or 0–24 h. The water prevented larvae from pupating too quickly [which occurs within 4 to 8 h (Frick et al., 1954)] and allowed sufficient numbers to be collected for experimentation. For adult experiments, larvae dropped onto soil for pupation, after which they were stored at 3 °C for 8–10 months before being used for experiments.

Laboratory experiments were conducted using a loose dry soil consisting of 1:1:1 (by volume) vermiculite:peat moss:sand (soil mix) wetted with deionized water to 20% moisture by weight (and confirmed using a Watch Dog data logger, Spectrum Technologies, Plainfield, IL) in clear plastic containers 11.5 cm diameter × 7.8 cm high. Volume and depth of soil were 150 cm^3 and 2 cm, respectively. *S. carpocapsae* (Sal strain), *S. feltiae*, and *S. intermedium* were grown in wax moth larvae, *Galleria mellonella* (L.), and infective juveniles (IJs) were harvested using methods presented by Kaya and Stock (1997). Depending on the experiment, either 50 or 100 IJs/cm^2 were applied onto the soil at 22 °C. Nematodes in 1 ml of water were evenly applied onto the soil surface using a pipette. Soil moisture was maintained by tightly sealing containers. Containers were held at 25–27 °C and 16 h light, conditions that mimic those in June during adult emergence and larval drop from cherries in the field.

2.2. General methods for laboratory larval experiments

In larval experiments, third instars (3.8–6.0 mm in length) that had dropped from fruit were added to soil treated with nematodes 10–30 min earlier to simulate larval drop from cherries onto treated ground. Unless noted, there were 5 replicates of 10 larvae each for all treatments and the controls. All treatments were replicated on the same day. Larvae and pupae were dissected to determine mortality after $\geqslant$7 days of exposure to IJs.

Larvae either were introduced directly from 23 °C water onto soil or were collected and added onto soil after they started to crawl in cups that had been drained of water 15–20 min earlier. For each introduction method, the burrowing time of 10 larvae was recorded.

2.2.1. Effect of nematode concentration on larval mortality

To determine the effects of nematode species and concentration on larval mortality, three tests were conducted with *S. carpocapsae*, *S. feltiae*, and *S. intermedium*, each at 50 and 100 IJs/cm^2. *S. intermedium* was not included in test 3. Fruit fly larvae were introduced directly from water onto soil in tests 1 and 2 and added onto soil after they had started to crawl in cups that had been drained of water in test 3.

2.2.2. Effect of interval between nematode application and addition of larvae

To assess the ability of nematodes to infect larvae at various times postapplication, larvae were dropped onto soil that had been treated with nematodes 0 (10–30 min), 2, 4, and 6 days earlier. Two tests were conducted with

[1] Referred to as *Steinernema feltiae* = *Neoaplectana carpocapsae* Weiser.

S. carpocapsae and *S. feltiae*, one at 50 and the other at 100 IJs/cm^2. In test 1, there were 4 or 8 replicates of 10 larvae for each treatment. In both tests, larvae were added onto soil after they had started to crawl in cups drained of water.

2.2.3. Effect of soil type on nematode activity

To evaluate soil type effects on nematode movement and larval mortality, *S. carpocapsae* and *S. feltiae* at 100 IJs/cm^2 were applied onto the soil mix and a Warden silt loam soil. The silt loam soil was collected from a commercial cherry orchard in Wapato, Washington, and is a well-drained (permeability of 1.5–5.1 cm/h), fine-textured soil with 8–15% clay and 0.5–1% organic matter (Lenfesty and Reedy, 1985). Soils were spread and dried in the sun for 6 h before being wetted to 20% moisture with deionized water. The numbers of nematodes seen on the surface during 2-min examinations were recorded at 30 min, 2, 4, and 6 h after applications. Larvae were added to soil after they had started to crawl in cups drained of water.

2.2.4. Effect of larvae being held in water on mortality caused by nematodes

To establish that mortality caused by nematodes was not affected by the larvae being held in water, a test comparing larvae that had been in water for 18–24 h after dropping from cherries with larvae that dropped directly onto soil mix was conducted. In the latter, larvae were collected every 4 h. In both, larvae were transferred to nematode-treated soil within 4 h of dropping from fruit. There were three replicates of *S. carpocapsae* and *S. feltiae* treatments at 50 IJs/cm^2 and three of a control, each with 10 larvae. Mortality was determined after 7 days.

2.3. Larval mortality in the field

The effect of nematodes on mortality of larvae under more natural climatic conditions was studied by adding 50 third-instar larvae onto natural silt loam soil (same type as in Section 2.2.3) with 20–25% moisture inside individual pail microcosms treated with either 50 or 100 IJs/cm^2 of *S. carpocapsae* or *S. feltiae* in a cherry orchard in Wapato, Washington. Pails were 15.5 cm diameter × 18.5 cm high and had been inserted into holes in the ground so that the tops were on level with the surface. Larvae were transferred directly from water onto soil 10 min after nematode applications. Sets of five pails (including the control) were spaced 25–30 cm apart underneath the north (shady) side of individual trees. One test was conducted on 18 and 21 June 2002 (data combined) with larvae that had been held in water for 0–48 h. Another was conducted on 24 June 2002 using larvae held in water for 0–48 h. Surface soil temperatures 1–4 h after nematode applications were 20–31 and 21–24 °C in tests 1 and 2, respectively. The experiment was arranged in a randomized block design, with four and three replicates per treatment in tests 1 and 2, respectively, blocking on individual trees. Each pail had a hole on the bottom covered with organdy to permit water passage. Tops of pails were covered with lids and organdy (to prevent larval escape). The soil was re-moistened 2–4 h after applications. Wet soil from extra pails was weighed, dried at 165 °C for 24 h, and then re-weighed to confirm soil moisture. Seven days after nematode applications, the pails were removed. Larvae and pupae were left in soil in the laboratory at 23 °C. Examination of soil for mortality required up to 8 weeks.

2.4. Effect of nematodes on pupae

The effect of nematodes on pupal mortality was determined by adding *S. carpocapsae* and *S. feltiae* at 100 IJs/cm^2 onto soil with pupae beneath or on the surface in the laboratory. Four tests were conducted, one with 1–3-week-old pupae that had been held at 23–26 °C before exposure (test 1), and the other three with 2-month-old pupae that had been stored for 1.5 months at 3 °C up to 1 day before tests. In tests 1 and 2, pupae were placed 1–2 cm below the soil surface. In tests 3 and 4, pupae were placed on the soil surface. Pupae were dissected and mortality was determined 14 days after treatment. There were five replicates of 10 pupae for each treatment and the control.

2.5. Effect of nematodes on adults

To determine the effect of nematodes on adult mortality, *S. carpocapsae*, *S. feltiae*, and *S. intermedium* at 50 and 100 IJs/cm^2 were applied onto soil containing 8–10-month-old pupae that had been placed 1–2 cm beneath the soil surface in the laboratory. Nematode applications were made 1 (test 1), 3 (test 2), or 7 days (test 3) before anticipated first emergence of flies (21 days after removal from cold). Adult emergence was followed for 14 days. Adults that died on the soil surface were removed daily and placed on moist filter papers inside sealed petri dishes at 22 °C to encourage nematode development. Three to four days later, the flies were dissected and the nematode numbers were recorded or approximated. Live adult flies were maintained inside pint-sized paper cartons with food (sugar and yeast) and water. Adults that died within 3 days were placed on moist filter papers and dissected. Each test consisted of five replicates of 100 pupae for each treatment and the control.

2.6. Statistical analyses

Data were analyzed with one-way, two-way, or randomized block analysis of variance (ANOVA), followed by Tukey's HSD test ($P < 0.05$). Percent mortality was

subjected to arcsine transformation before analyses. Data were analyzed using the Statistical Analysis System (SAS Institute, 2001).

3. Results

3.1. Behavior of fly larvae on soil

Exposure times of larvae to nematodes on the soil surface were relatively short. When transferred directly from water onto soil, larvae started to move within 5 min and all burrowed completely into the soil after 14–22 min. When larvae were added onto soil only after they had started to crawl in cups drained of water, they were more active and burrowed into the soil after 3–7 min.

3.2. Effect of nematode concentration on larval mortality

In tests 1 and 2, *S. carpocapsae* and *S. feltiae* treatments at low and high concentrations resulted in 100% larval mortality (Table 1). *S. intermedium* in tests 1 and 2 also caused relatively high mortality, at 54–96% (test 1: $F_{6,28} = 38.68$, $P < 0.0001$; test 2: $F_{6,28} = 26.49$, $P < 0.0001$). In test 3, *S. carpocapsae* and *S. feltiae* caused lower mortalities than in previous tests, but both caused significant mortality ($F_{4,20} = 18.22$, $P < 0.0001$) and were equally effective within concentrations. In tests 1, 2, and 3, 36–46%, 72–80%, and 0–4%, respectively (test 1: $F_{6,28} = 4.57$, $P = 0.0024$; test 2: $F_{6,28} = 3.86$, $P = 0.0062$; test 3: $P > 0.05$), of the larvae exposed to nematodes died before pupating (Table 1). Dissection of larvae and pupae revealed the presence of nematodes, but nematodes inside both insect stages were dead, deteriorated, and not identifiable in many cases.

3.3. Effect of interval between nematode application and addition of larvae

There were significant time × treatment interactions (two-way ANOVA, $P < 0.05$), so one-way ANOVA was separately conducted between species within days and within species among days. In test 1, *S. carpocapsae* and *S. feltiae* were nearly equal in effectiveness on all days (Table 2). In test 2, *S. carpocapsae* was more effective than *S. feltiae* as days progressed. *S. carpocapsae* generally caused greater mortality than *S. feltiae* (Table 2), with nearly all fly deaths occurring in the pupal stage.

3.4. Effect of soil type on nematode activity

More *S. carpocapsae* than *S. feltiae* were seen on the surfaces of both soil types at 0.5–6 h (Table 3) (repeated-measures ANOVA; soil mix: $F_{1,8} = 350.88$, $P < 0.0001$; silt loam soil: $F_{1,8} = 28.94$, $P = 0.0007$). Despite the differences in the numbers seen, the two species caused equally high mortality of larvae within soil types (soil mix: $F_{2,12} = 30.96$, $P < 0.0001$; silt loam soil: $F_{2,12} = 14.52$, $P < 0.0006$). After 6 h, 2.7 and 7.3% of larvae had not penetrated the surfaces of the soil mix and silt loam soil, respectively.

3.5. Effect of larvae being held in water on mortality caused by nematodes

The mortality of larvae that had been in water for 24 h and those that dropped directly onto soil was 100% for *S. carpocapsae* and *S. feltiae* treatments. There was no mortality in the controls ($P < 0.05$).

3.6. Larval mortality in the field

Mortalities caused by both nematodes in the field were equal and usually higher than in the controls (Table 4), when calculated based on total numbers of larvae at the start of tests (test 1: $F_{4,12} = 9.15$, $P = 0.0013$; test 2: $F_{4,8} = 5.48$, $P = 0.0200$) or on numbers of larvae and pupae that were recovered (test 1: $F_{4,12} = 12.10$, $P = 0.0004$; test 2: $F_{4,8} = 5.47$, $P = 0.0202$). Recovery rates of dead and live pupae in controls were 69 and 63%, compared with rates in nematode treatments of 47 and 45% (both species combined) in tests 1 and 2, respectively.

Table 1
Percent mortality of *R. indifferens* exposed as third-instar larvae to two concentrations of three *Steinernema* species in the laboratory

Treatment	Overall percent mortality			Percent dead as larvae[a]		
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
Control	6.0 ± 4.0a[b,c]	22.0 ± 9.7a[b,c]	4.0 ± 2.4a[b,c]	6.0 ± 4.0a[b,c]	22.0 ± 9.7a[a,b]	0 ± 0a[b,c]
S. carpocapsae—50[d]	100 ± 0b	100 ± 0b	62.0 ± 6.6bc	46.0 ± 10.3b	74.0 ± 11.7b	2.0 ± 2.0a
S. carpocapsae—100[d]	100 ± 0b	100 ± 0b	84.0 ± 6.8b	42.0 ± 8.6b	74.0 ± 6.8b	0 ± 0a
S. feltiae—50	100 ± 0b	100 ± 0b	54.0 ± 12.1c	44.0 ± 11.2b	72.0 ± 8.0b	4.0 ± 4.0a
S. feltiae—100	100 ± 0b	100 ± 0b	78.0 ± 4.9bc	42.0 ± 5.8b	80.0 ± 8.4b	0 ± 0a
S. intermedium—50	66.0 ± 11.7c	96.0 ± 4.0b	–	36.0 ± 8.1b	80.0 ± 8.4b	–
S. intermedium—100	54.0 ± 12.1c	92.0 ± 8.0b	–	44.0 ± 9.3b	80.0 ± 7.1b	–

[a] Many larvae exposed to nematodes died as pupae.
[b] Mean ± SE.
[c] Means followed by the same letter within columns are not significantly different (Tukey's HSD test, $P > 0.05$).
[d] No. of infective juveniles/cm^2.

Table 2
Percent mortality of *R. indifferens* exposed as third-instar larvae to two concentrations of two *Steinernema* species in soil 0–6 days post-nematode application in the laboratory

Time (days)	*N*	Control	*S. carpocapsae*	*S. feltiae*
		Test 1—50 infective juveniles/cm^2		
0[a]	8	4.3 ± 2.0a(a)[b,c]	78.6 ± 5.5a(b)[b,c]	87.5 ± 3.4a(b)[b,c]
2	4	7.5 ± 4.8a(a)	92.5 ± 2.5a(b)	52.5 ± 6.3b(c)
4	4	10.0 ± 0.0a(a)	95.0 ± 5.0a(b)	92.5 ± 2.5a(b)
6	4	25.0 ± 9.6a(a)	77.5 ± 16.5a(b)	70.0 ± 4.1b(b)
		Test 2—100 infective juveniles/cm^2		
0[a]	5	8.0 ± 5.8a(a)[b,c]	90.0 ± 5.5a(b)[b,c]	90.0 ± 5.5a(b)[b,c]
2	5	10.0 ± 4.5a(a)	92.0 ± 3.7a(b)	50.0 ± 7.7b(c)
4	5	24.0 ± 12.9a(a)	100.0 ± 0.0a(b)	42.0 ± 8.0b(a)
6	5	2.0 ± 2.0a(a)	84.0 ± 8.1a(b)	40.0 ± 14.8b(c)

[a] Larvae were placed on the soil 10–30 min before nematodes were applied.
[b] Mean ± SE.
[c] Means followed by the same letter within columns (day effect) and rows (letters inside parentheses, species effect) are not significantly different (Tukey's HSD test, $P > 0.05$).

Table 3
Numbers of two *Steinernema* species seen in different soil types at 0.5–6 h post-nematode application and mortality of *R. indifferens* exposed as third-instar larvae to 100 infective juveniles/cm^2

Time (h) after application	*N*	No. of nematodes seen on surface during 2 min			
		Soil mix[a]		Silt loam soil	
		S. carpocapsae	*S. feltiae*	*S. carpocapsae*	*S. feltiae*
0.5	5	24.8 ± 3.4[b]	0.0 ± 0.0[a]	57.0 ± 14.4[b]	5.8 ± 0.7[b]
2	5	12.2 ± 1.7	1.2 ± 0.4	4.6 ± 1.2	3.8 ± 1.0
4	5	10.2 ± 0.6	2.2 ± 0.4	9.2 ± 2.7	3.4 ± 1.2
6	5	10.6 ± 1.1	2.6 ± 0.7	14.6 ± 1.9	3.0 ± 0.8
Treatment	*N*	Percent mortality Soil mix[a]	*N*	Silt loam soil	
Control	5	4.0 ± 2.0a[b,c]	5	20.0 ± 7.0a[b,c]	
S. carpocapsae	5	80.0 ± 3.0b	5	78.0 ± 10.0b	
S. feltiae	5	66.0 ± 10.0b	5	74.0 ± 7.0b	

[a] 1:1:1 (by volume) vermiculite:peat moss:sand.
[b] Mean ± SE.
[c] Means followed by the same letter within columns are not significantly different (Tukey's HSD test, $P > 0.05$).

Table 4
Percent mortality of *R. indifferens* exposed as third-instar larvae to two concentrations of two *Steinernema* species in silt loam soil in microcosms in a cherry orchard

Treatment	*N*	Test 1—18, 21 June		*N*	Test 2—24 June	
		% of totals[a]	% of recovered		% of total	% of recovered
Control	4	36.0 ± 3.7a[b,c]	7.3 ± 1.8a[b,c]	3	48.0 ± 1.2a[b,c]	16.8 ± 3.4a[b,c]
S. carpocapsae—50[d]	4	79.0 ± 2.1b	53.5 ± 3.5b	3	59.3 ± 4.7ab	33.9 ± 2.5ab
S. carpocapsae—100[d]	4	67.5 ± 5.7ab	40.7 ± 4.7b	3	76.0 ± 7.0ab	56.8 ± 11.2b
S. feltiae—50[d]	4	76.5 ± 6.6b	57.4 ± 10.7b	3	80.0 ± 7.2b	46.0 ± 8.8ab
S. feltiae—100[d]	4	85.5 ± 7.5b	62.4 ± 11.5b	3	80.0 ± 3.5b	52.8 ± 7.7b

Note. There were 50 larvae/replicate.
[a] Larvae and pupae not recovered were assumed to have died and disintegrated in the soil.
[b] Mean ± SE.
[c] Means followed by the same letter within columns are not significantly different (Tukey's HSD test, $P > 0.05$).
[d] No. of infective juveniles/cm^2.

3.7. Effect of nematodes on pupae

Neither *S. carpocapsae* nor *S. feltiae* caused significant mortality in completely formed pupae in the four tests ($P > 0.05$). Mortality ranged from 0 to 21.1%. No nematodes were positively identified inside the puparia.

3.8. Effect of nematodes on adults

The three nematode species generally did not affect adult emergence, but there were differences in their infectivity rates of adults that did emerge (Table 5). *S. carpocapsae* infected the highest percentage of emerged adults (test 1: $F_{3,16} = 17.20$, $P < 0.0001$; test 2: $F_{3,16} = 46.02$, $P < 0.0001$; test 3: $F_{3,16} = 3.29$, $P = 0.0479$ [although analysis with Tukey's HSD test indicated no mean separation in test 3]), followed by *S. feltiae* and *S. intermedium*. *S. carpocapsae* also produced the highest numbers of nematodes within a host: 1 to >200 and 1 to >400 *S. carpocapsae*, 2 to >100 and 1 to 145 *S. feltiae*, and 5 and 1 to 10 *S. intermedium* were found inside adult flies in tests 1 and 2, respectively. All nematode stages (IJs to adults), dead and alive, were recovered, indicating reproduction occurred inside flies.

Of 32 sexed adults infected with *S. carpocapsae* in tests 1 and 2, 44% were males and 56% were females (50:50 sex ratio for all emerged flies). In these tests, of 30 adults infected by *S. carpocapsae*, 57% died on the soil surface; of those that left the soil and died in cages within 3 days ($n = 21$), 62% were infected. These figures were 71 ($n = 7$) and 7% ($n = 30$) for *S. feltiae* and 50 ($n = 4$) and 8% ($n = 24$) for *S. intermedium*. No infected adults were found beneath the soil surface.

4. Discussion

The results indicate that larvae of *R. indifferens* are highly susceptible to steinernematid nematodes, pupae are not susceptible, and that adults are marginally to moderately susceptible. Factors that cause differences among insect stages and nematode species effectiveness need to be determined. However, there are several explanations for the results.

The greater susceptibility of the larvae was probably caused by a combination of their duration in soil, their high activity in soil and CO_2 output, and perhaps their larger body openings, which are the portals of entry for steinernematids (Ishibashi and Kondo, 1990). *S. carpocapsae* and *S. feltiae* probably penetrate *R. indifferens* larvae via the anus as they do in larvae of the agromyzid leafminer fly, *Liriomyza trifolii* (Burgess) (LeBeck et al., 1993), and of the house fly, *Musca domestica* L. (Renn, 1998), respectively, and not the spiracles. *S. carpocapsae* was generally the most effective of the three nematodes in laboratory studies. Whether the small differences in size—IJs of *S. carpocapsae* measure 20–30 μm, *S. feltiae* 22–29 μm, and *S. intermedium* 25–32 μm in width (Poinar, 1990)—can result in differences in infectivity needs to be determined. The reduction in larval mortality and the few deaths that occurred as larvae in test 3 may have been a result of larvae being close to pupation when exposed to nematodes. It was concluded that holding the larvae in water prior to nematode exposure did not increase the susceptibility of larvae to nematode infection in any test.

The foraging strategies of the nematode species differ, but how these strategies affect differential mortality is unclear. *S. carpocapsae* displays a "nictation" or ambusher foraging strategy in which it stays on the soil

Table 5
Percent emergence and infection of emerged adult *R. indifferens* exposed to 100 infective juveniles/cm^2 of three *Steinernema* species in soil in the laboratory

Treatment	Percent adult emergence		
	Test 1[a]	Test 2[b]	Test 3[c]
Control	14.8 ± 1.5a[d,e]	30.4 ± 2.2a[d,e]	15.2 ± 4.0a[d,e]
S. carpocapsae	7.6 ± 2.1b	21.6 ± 2.5a	15.0 ± 3.9a
S. feltiae	15.0 ± 0.7a	30.0 ± 3.4a	14.8 ± 1.2a
S. intermedium	15.4 ± 2.4a	19.8 ± 3.0a	19.8 ± 3.7a
	Percent of emerged adult flies infected		
Control	0 ± 0a[d,e]	0 ± 0a[d,e]	0 ± 0a[d,e]
S. carpocapsae	53.1 ± 12.5b	21.7 ± 3.7b	11.3 ± 5.8a
S. feltiae	4.1 ± 1.7a	6.1 ± 1.2c	3.2 ± 2.0a
S. intermedium	6.7 ± 2.9a	0.7 ± 0.7a	0 ± 0a

[a] Nematodes applied 1 day before adult emergence.
[b] Nematodes applied 3 days before adult emergence.
[c] Nematodes applied 7 days before adult emergence.
[d] Mean ± SE.
[e] Means followed by the same letter within columns are not significantly different (Tukey's HSD test, $P > 0.05$).

surface waiting for its host, whereas *S. feltiae* displays an intermediate ambusher–cruiser foraging strategy in which it waits for or seeks its host (Lewis, 2002). (The foraging strategy of *S. intermedium* has not been reported.) Because *R. indifferens* larvae burrowed into the soil mix in 3–22 min, *S. feltiae* should have caused greater mortality than *S. carpocapsae* by seeking larvae beneath the surface. This was not seen in our laboratory study, suggesting the small test containers and soil type negated behavioral differences.

Many *R. indifferens* exposed as larvae to nematodes died as pupae similar to previous results by Patterson Stark and Lacey (1999). In addition, larvae of *C. capitata*, *Dacus cucurbitae* Coquillett, and *D. dorsalis* Hendel (all Tephritidae) exposed to *S. carpocapsae*[1] (Lindegren and Vail, 1986) died as pupae. Late third-instar larvae and prepuparia of *L. trifolii* died an average of 7.4 and 15 h, respectively, after penetration by *S. carpocapsae* (LeBeck et al., 1993).

In laboratory tests, *S. carpocapsae* and *S. feltiae* at both 50 and 100 IJs/cm^2 caused high mortality. Both concentrations were apparently sufficient for high encounters between the nematodes and the mobile larvae. In the field, *S. carpocapsae*[1] at a concentration of 5000 IJs/cm^2 was more effective than at 150–500 IJs/cm^2 against larvae of *C. capitata* when measured by adult fly emergence (Lindegren et al., 1990).

Both *S. carpocapsae* and *S. feltiae* were effective in killing larvae up to 6 days after application. The energy reserves in *S. carpocapsae* were clearly sufficient for at least 6 days to allow the nematodes to cause high infection rates in *R. indifferens*. *S. carpocapsae* was slightly more effective than *S. feltiae* following application of IJs, perhaps because *S. carpocapsae* used fewer reserves, allowing it to be more infective over longer periods in soil.

More *S. carpocapsae* were found on the surface of the soil mix and silt loam soils than *S. feltiae* at all times, suggesting greater vertical movement by the latter, which is consistent with their foraging strategies. Despite this difference and the slightly lower penetration by larvae into the silt loam soil, the soil types tested did not affect the ability of either species to find and infect larvae. This supports work that showed *S. carpocapsae* was nearly 100% pathogenic against *G. mellonella* larvae on sand, sandy loam, and clay loam soils during the first week following application (Kung et al., 1990). Over time, however, higher clay soils seemed to reduce nematode movement (Georgis and Poinar, 1983) and survival (Kung et al., 1990).

The highest mortality caused by both nematode species in the field was only slightly lower than that seen in laboratory tests. Mortalities in the controls and nematode treatments in the field tests were higher than expected. Larvae used in these tests had been held in water contaminated with juice from cherries (on screens above the water) for 0–24 or 0–48 h in warm temperatures during June. The fouling of the water possibly reduced the vigor of some larvae. However, because larvae from the same water were used in the controls and all treatments, nematodes were clearly the cause of the high mortality. Larvae in the field were also subject to natural mortality and decomposition, factors encountered less frequently in the laboratory, making total recovery problematic.

Because of the zero tolerance for *R. indifferens* in commercial cherry orchards, a successful fly control program should increase larval mortality to levels greater than that seen in the field tests. To achieve this, >100 IJs/cm^2 may be needed, which was done with *S. carpocapsae*[1] against *C. capitata* (Lindegren et al., 1990). Applications during cooler times of the day should increase efficacy, especially if they coincide with larval drop from trees. Adult *R. indifferens* emerge in the cool morning hours (Frick et al., 1954), and this probably also occurs with larvae from fruit. Larval walnut husk flies, *Rhagoletis completa* Cresson, drop from nuts during the cooler and more humid times of the day (Boyce, 1934).

Unlike the larvae, the pupae of *R. indifferens* were not susceptible to nematodes. Similarly, only 0–1.0% of *A. suspensa* pupae was infected by *S. carpocapsae* (Beavers and Calkins, 1984). The openings on the spiracles of the puparia may be too small for nematodes to enter. Nematodes were found adhering to the spiracles and posterior ends of *R. indifferens* puparia (Patterson Stark and Lacey, 1999), but entry by nematodes could not be confirmed by dissections. *Liriomyza trifolii* puparia >1 h postpupation were not susceptible to *S. carpocapsae* infection, possibly because of closure of anal and oral apertures (LeBeck et al., 1993).

Although overall nematode infections of adult *R. indifferens* were relatively low, the results specifically showed that *S. carpocapsae* was more effective than *S. intermedium* and *S. feltiae* against the adults. Perhaps its slightly smaller size allowed it to penetrate the mouth or anal aperture more easily. These are the only routes of penetration for *S. feltiae* in adult *M. domestica* (Renn, 1998). Because nematode species effects were greater with adults than with larvae, it is also possible that the bacterium associated with *S. carpocapsae*, *Xenorhabdus nematophila* (Poinar and Thomas), and that associated with *S. feltiae* and *S. intermedium*, *X. bovienii* (Akhurst) (Akhurst and Boemare, 1990), elicit different immune responses by larval and adult flies. Previous studies also showed that *S. carpocapsae*[1] infects adult subtropical tephritid flies (Beavers and Calkins, 1984; Ghally, 1988; Lindegren and Vail, 1986; Poinar and Hislop, 1981), but none has reported infection of adult flies by *S. feltiae* and *S. intermedium*.

The relatively low infection rates of adult *R. indifferens* even by *S. carpocapsae* may result from the short

time spent by adults in or on the soil. Most *S. carpocapsae* and *S. feltiae* probably attacked adults that were crawling up through the soil and not those resting on the surface, where body surface area contact with nematodes was lower. Similar to the present findings, *S. carpocapsae*[1] applications made on soil resulted in only 0.2% infection of adult *C. capitata* (Lindegren and Vail, 1986). In contrast, when exposed to nematodes inside petri dishes for longer periods, *S. carpocapsae*[1] caused 45–100% mortality of adult *C. capitata* (Ghally, 1988) and 70–92% mortality of 2–5-day-old adult *A. suspensa* (Beavers and Calkins, 1984). In the former study, 1000 and 50 nematodes/adult caused 100% mortality after 24 and 48 h, respectively. Repeated contact with high numbers of nematodes, therefore, may be the key factor in achieving high infection rates of adult tephritid flies, suggesting that infections of adult *R. indifferens* would have been higher if the flies had stayed in soil longer.

The high pathogenicity of *S. carpocapsae* and *S. feltiae* against *R. indifferens* larvae and their persistence in soil as well as efficacy in different soil types indicate both nematodes hold promise as effective biological control agents of flies in isolated and abandoned lots or in yards of homeowners. Studies of nematode behavior and survival under cherry trees and of fly larval ecology are needed to further evaluate the potential of using nematodes for the control of *R. indifferens*.

Acknowledgments

We thank Heather Headrick, Angie Bosma, Vicki Baca, Julie Schlaman, Marisol Laddrout, Wendy Heilman, Matt Heilman, and Pete Chapman for technical assistance, and Don Hostetter and David Shapiro-Ilan for reviewing earlier drafts of the manuscript. We also thank Zdenek Mrácek, Czech Academy of Sciences, for supplying *S. intermedium* and Clifford Plath and Jim Hargrove for allowing us the use of their cherry orchard. This research was partially funded by the Washington Tree Fruit Research Commission.

References

Akhurst, R.J., Boemare, N.E., 1990. Biology and taxonomy of *Xenorhabdus*. In: Gaugler, R., Kaya, H.K. (Eds.), Entomopathogenic Nematodes in Biological Control. CRC Press, Boca Raton, FL, pp. 75–90.

AliNiazee, M.T., 1974. The western cherry fruit fly, *Rhagoletis indifferens* (Diptera: Tephritidae) I. Distribution of the diapausing pupae in the soil. Can. Entomol. 106, 909–912.

Beavers, J.B., Calkins, C.O., 1984. Susceptibility of *Anastrepha suspensa* (Diptera: Tephritidae) to steinernematid and heterorhabditid nematodes in laboratory studies. Environ. Entomol. 13, 137–139.

Boyce, A.M., 1934. Bionomics of the walnut husk fly, *Rhagoletis completa*. Hilgardia 8, 363–579.

Frick, K.E., Simkover, H.G., Telford, H.S., 1954. Bionomics of the cherry fruit flies in eastern Washington. Wash. Agric. Expt. Sta. Tech. Bull. 13, 66 pp.

Georgis, R., Poinar Jr., G.O., 1983. Effect of soil texture on the distribution and infectivity of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae). J. Nematol. 15, 308–311.

Ghally, S.E., 1988. Pathogenicity of the nematode *Steinernema feltiae* Filipjev in relation to different insect hosts. J. Egypt. Soc. Parasitol. 18, 297–304.

Ishibashi, N., Kondo, E., 1990. Behavior of infective juveniles. In: Gaugler, R., Kaya, H.K. (Eds.), Entomopathogenic Nematodes in Biological Control. CRC Press, Boca Raton, FL, pp. 139–150.

Kaya, H.K., Stock, S.P., 1997. Techniques in insect nematology. In: Lacey, L.A. (Ed.), Manual of Techniques in Insect Pathology. Academic Press, London, pp. 281–324.

Kung, S.P., Gaugler, R., Kaya, H.K., 1990. Soil type and entomopathogenic nematode persistence. J. Invertebr. Pathol. 55, 401–406.

LeBeck, L.M., Gaugler, R., Kaya, H.K., Hara, A.H., Johnson, M.W., 1993. Host stage suitability of the leafminer *Liriomyza trifolii* (Diptera: Agromyzidae) to the entomopathogenic nematode *Steinernema carpocapsae* (Rhabditida: Steinernematidae). J. Invertebr. Pathol. 62, 58–63.

Lenfesty, D., Reedy, T.E., 1985. Soil survey of Yakima County area, Washington. USDA-Soil conservation service, 345 pp.

Lewis, E.E., 2002. Behavioural ecology. In: Gaugler, R. (Ed.), Entomopathogenic Nematology. CABI Publishing, Wallingford, UK, pp. 205–223.

Lindegren, J.E., Vail, P.V., 1986. Susceptibility of Mediterranean fruit fly, melon fly, and oriental fruit fly (Diptera: Tephritidae) to the entomogenous nematode *Steinernema feltiae* in laboratory tests. Environ. Entomol. 15, 465–468.

Lindegren, J.E., Wong, T.T., McInnis, D.O., 1990. Response of Mediterranean fruit fly (Diptera: Tephritidae) to the entomogenous nematode *Steinernema feltiae* in field tests in Hawaii. Environ. Entomol. 19, 383–386.

Patterson Stark, J.E., Lacey, L.A., 1999. Susceptibility of western cherry fruit fly (Diptera: Tephritidae) to five species of entomopathogenic nematodes in laboratory studies. J. Invertebr. Pathol. 74, 206–208.

Poinar Jr., G.O., 1990. Taxonomy and biology of Steinernematidae and Heterorhabditidae. In: Gaugler, R., Kaya, H.K. (Eds.), Entomopathogenic Nematodes in Biological Control. CRC Press, Boca Raton, FL, pp. 23–61.

Poinar Jr., G.O., Hislop, R.G., 1981. Mortality of Mediterranean fruit fly adults (*Ceratitis capitata*) from parasitic nematodes (*Neoaplectana* and *Heterorhabditis* spp.). IRCS Med. Sci: Biochem.; Devel. Biol. Med.; Microbiol. Parasitol. Infect. Dis. 9, 641.

Renn, N., 1998. Routes of penetration of the entomopathogenic nematode *Steinernema feltiae* attacking larval and adult houseflies (*Musca domestica*). J. Invertebr. Pathol. 72, 281–287.

SAS Institute. 2001. SAS/STAT User's Guide, version 8. Cary, NC.

Shapiro-Ilan, D.I., Gouge, D.H., Koppenhöfer, A.M., 2002. Factors affecting commercial success: case studies in cotton, turf and citrus. In: Gaugler, R. (Ed.), Entomopathogenic Nematology. CABI Publishing, Wallingford, UK, pp. 333–355.

Zwick, R.W., Fields, G.J., Kiigemagi, U., 1975. Dimethoate for control of western cherry fruit fly on sweet cherries in Oregon. J. Econ. Entomol. 69, 383–385.

Zwick, R.W., Jones, S.C., Peifer, F.W., Every, R.W., Smith, R.L., Thienes, J.R., 1970. Malathion ULV aerial applications for cherry fruit fly control. J. Econ. Entomol. 63, 1693–1695.